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STUDY OF JUVENILE AND ADULT GROWTH, AND BEHAVIOURAL CHARACTERISTICS OF *POECILOCERUS PICTUS* (FABRICIUS) FEEDING ON *CALOTROPIS GIGANTEA* UNDER LABORATORY CONDITIONS

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Insects are reared to study various aspects of their life cycle, behaviour and metabolism and for experimentation with insecticides. In the present investigation, the newly hatched nymphs of *Poecilocerus pictus* (Fabricius) feeding on *Calotropis gigantea* were reared to adulthood in a laboratory to evaluate the developmental and behavioural characteristics. On the basis of the experimental observations, it was concluded that under constant laboratory conditions they could be grown and maintained for a longer period of time with maximum growth in length and weight. Under optimum laboratory conditions, a strong correlation was observed between length and weight, in addition to extended longevity and shortened nymphal periods.

Key words: *Poecilocerus pictus*, longevity, feeding, aggressiveness, nymphal periods, correlation coefficient

INTRODUCTION

Many industries need insects for research projects as they develop insecticides, or insect resistant plant varieties for organic farming and similar needs. A few species of grasshopper sometimes occur in large numbers and cause serious damage to vegetable crops and landscape ornamentals. One species most commonly causing damage is the Painted Grasshopper *Poecilocerus pictus* (Fabricius, 1775) (Orthoptera: Acridoidea: Pyrgomorphidae), distributed in South East Asia; it is a large grasshopper. The most noticeable feature of this grasshopper is its long jumping hind legs, which enable it to leap more than 20 times its body length. It mainly feeds on the shrubby plants of Family Asclepiadaceae – *Calotropis procera* and *Calotropis gigantea*. By the time the insects reach adulthood the *Calotropis* sp. is completely denuded, and the grasshopper migrates to adjacent supplementary host plants. *P. pictus* is an economic pest in Pakistan and India where it is reported to damage a number of food plants, including aubergine, citrus, cucurbits, potatoes and tomatoes (Garod 2009). Sayed *et al.* (1994) studied the effects of different food plants on the rate of consumption, development and survival of *Poecilocerus pictus* under laboratory conditions. The results indicated that the rate of development of *P. pictus* was faster on *Calotropis* sp. followed by cotton, nerium, champa, pomegranate, maize, jamun, tomato, rose sesame, shoeflower, sugarcane, and lemon. Incidental observations indicate that *P. pictus* are not easy to kill with insecticides, once they become large. One has to ensure that the insecticide is sprayed directly on the insects as the insecticide residue remaining on sprayed plants is not adequate to kill the grasshoppers.

Poecilocerus pictus sequesters and stores secondary metabolites – cardenolides – obtained from *Calotropis* sp., its food plant, in the secretion of the defensive glands and other parts of the body. Cardenolide content in different tissues of gravid females has been analysed, and statistically significant differences in its levels have been detected in the metathoracic scent gland, ovary and egg, which were found to sequester higher concentrations of cardenolides (Pugalenthi and Livingstone 1995). The cardenolides are not toxic to the grasshoppers, but they make them unpalatable to predators, and become an important part of a grasshopper's defence system.

To study plant-insect relation and tolerance of the insect to the toxins in the evolutionary path and its regulation to avoid serious damage to crop and ornamental plants, it is important to rear insects under laboratory conditions for a longer period, as their availability in the wild/natural habitat is restricted from late July to early November. In the present investigation, we reared the *Poecilocerus pictus* (Fabricius) in the laboratory to maintain a year round supply and to get a disease-free population. Information on the life cycle of *P. pictus* are important parameters to rear the insects in laboratory. While rearing this species in laboratory significant parameters within its life cycle, such as oviposition, developmental biology of immature stages, adult longevity, behaviour and growth in terms of length and weight were evaluated against their growth and behaviour in natural habitat.

MATERIAL AND METHODS

Collection and rearing

Adult *P. pictus* and their nymphs were collected during August-October (2006-2009) from an area located on the outskirts of Mumbai from an infested *Calotropis gigantea*. The adults and nymphs were separated and kept in separate cages. Adult insects collected directly from their natural habitat were labelled as 'Group I', and the nymphs reared to adulthood in the laboratory as 'Group II'.

Sexes were identified and their growth parameters (length and weight) were noted. Newly emerged nymphs were caged over moist soil in the laboratory. A standard system was developed for routine maintenance of *P. pictus*.

Sufficiently ventilated plastic baskets (45×30×45 cm) with a fine mesh structure, offering protection and excellent light transmission, were used as growth chambers. Two wooden rods were placed horizontally inside these chambers to support the moulting stage of the insects. Chambers were provided with 8 to 10 cm of soil bed (mixture of moist soil or clay and sand), which provided moisture as well as surfaces on which to rest and oviposit. There was an opening (45 x 30 cm) covered with polyvinyl sheath for introducing food. Temperature fluctuations and relative humidity within rearing chambers were measured every day throughout the period of growth using a thermometer and thermo-hydrograph respectively. The nymphs were exposed to photoperiod of 12 to 14 hours per day by keeping these chambers in maximum daylight.

Nymphs were fed on fresh leaves of *C. gigantea*. The amount and time of feeding was standardized by trial and error method, after observing their feeding behaviour; 7-9 gm (wet weight) of fresh and thoroughly washed leaves per chamber, thrice a day, after an interval of 8 hrs. Leaves were kept away from direct sunlight to avoid drying.

To study the developmental stages, 8 sets of 5 to 6 newly hatched first instar nymphs were placed in the growth chamber. As sexual dimorphism was not obvious in nymphal stages, they were tagged with whitener (as 1, 2, 3...). The sexes were identified only after maturation. These nymphs were left undisturbed to feed, moult, and eventually metamorphose into adults. After every moult the instar was renumbered. All the stages were observed daily till maturation and further till death to determine longevity. Exuviae were removed as they appeared and the duration of each instar with total number of instars and nymphal periods in days were recorded.

Within 24 hrs of the last moult, adults were separated. Batches of 5 to 6 males and 3 females were placed separately in well-ventilated 5 rearing chambers having similar conditions maintained as in growth chambers. Pre-oviposition, oviposition and post-oviposition periods in adult females, and courtship and mating behaviour in males were studied. Preliminary observations of mating and egg-laying behaviour were carried out by observing the individual until the end of the desired behaviour (Ganehiarachchi and Fernando 2006) and duration of time for the behaviour was recorded (n = 15).

Morphology and Morphometry

Morphological features of the eggs, nymphs and adults were examined under magnifying lens. Length and breadth of each egg pod, egg, and nymph from group 'I' and adults from both the groups were measured using dividers and millimeter scale (Ganehiarachchi and Fernando 2006). Weight of fully-grown adults from both the groups was also recorded; they were placed in closed pre-weighed Petri-plates, to restrict their movements while weighing. Weights were recorded using a digital analytical balance calibrated with IDEMI certified weights.

Perception of odour by human volunteers

P. pictus has a noxious odour as well as bright yellow bands on its body which probably act as repellent for predators. *P. pictus* odour is more noxious than that of *Calotropis* sp. The degree of noxiousness of the odour was evaluated as described elsewhere (Idowu and Idowu 1999). Ten insects at different developmental stages were placed in different conical flasks covered with foil. The flasks were numbered 1-3 corresponding to (1) instar 2, (2) instar 5, and (3) adult stages. The flasks were thoroughly shaken before presentation. The order of presentation of flasks was changed for each volunteer. A time gap of 30 minutes was allowed between presentations of samples. The perception of odour by 38 human volunteers was recorded as follows:

1) Very Strong: immediate response
2) Strong: within 5 - 15 seconds
3) Weak: within 20 - 25 seconds
4) No effect: The volunteer did not perceive the odour

The intensity of the odour was allotted 3, 2, 1 and 0 scores respectively.

Statistical analysis

Results obtained were statistically analyzed using student's t-test and expressed as Mean ±SD of the experimental observations.

For all comparisons, significance was determined at $P \leq 0.05$. Linear regression (Curxpt software) and Correlation coefficient between length and weight of grasshoppers in Group I and II were analysed.

Fig. 1: Nymph *Poecilocerus pictus*

Fig. 2: Adult *Poecilocerus pictus*

RESULTS

A. Behaviour patterns

1. Defensive Behaviour

Both nymphs and adults showed defensive behaviour. Their aposematic coloration informs potential predators that they are poisonous or unpalatable. The immature grasshopper differs in appearance from the adults. Nymphs (Fig. 1) typically are completely bright yellow with black and red spots all over the body, whereas adults (Fig. 2) have bright yellow and blue stripes alternately on whole body, including antennae and legs. Hind wings of adult grasshoppers are bright scarlet-red to orange, in sharp contrast to the often drab brown with blackish blue mosaic pattern of the forewings. When disturbed, they take to the air, diverting attention to the brightly coloured and flashy hind wing, and disappear from sight by folding their wings, landing, and cryptically blending into the background. Nymphs (as they cannot fly), and sometimes adults, hide behind leaves and rod placed inside the chamber or hop and drop themselves from whatever they are holding and hop away from the site. Nymphs forcibly eject a secretion stored in the salivary system, closely associated with the crop and midgut, several times, over 30 cm, in the direction of the disturbance. The Nymphs simultaneously contract the abdomen to force air out of the spiracles accompanied with a peculiar sound. In case of adults, the secretion flows down the sides of the body along lateral grooves into the spiracles of the second abdominal segment where it mixes with air to form a repellent froth.

Table 1: Perception of the odour of the body of *P. pictus* by human volunteers

Sample	Percentage of respondents stating how they perceive the odour of *P. pictus* n=38 (100%)			
	Very strong	Strong	Weak	No smell
2nd instar	13.16*	31.58*	52.63*	2.63*
5th instar	21.05**	47.37**	31.58*	0.00 (NS)
Adult	31.58**	65.79**	2.63*	0.00 (NS)

*: Statistically Significant, **: Highly Significant, NS: Statistically not significant

Perception of odour of *P. pictus* by human volunteers: The odour of the secretion was instantly recognized by human volunteers as strong and repulsive. The response of the volunteers indicated that a significantly different odour (t-test, $P>0.005$, Table 1) is produced by *P. pictus* which is low in 2nd instar, intermediate in 5th, and high in the adult. Incidental observation also showed that the secretion led to allergic reactions, such as redness and rash on skin, at times swelling and eye irritation (data not shown).

2. Feeding

Nymphs of *P. pictus* were successfully reared on *Calotropis gigantea* in the laboratory. Wet and fresh leaves were preferred by nymphs over dry and stored leaves, as fresh leaves were juicy with latex. Positive reaction towards odour of food and light was also observed. Average food consumption of male and female in nymphal period was 2 gm and 4.45 gm per individual per day respectively. Feeding rate increased during day time (between 10:00 and 12:00 hrs). Feeding rate was highest during the second instar in both sexes, males: 2.88 gm and females: 5.15 gm per day per individual.

3. Moulting

Temperature of 29°C ±3 and 40-50% of relative humidity was noted. All embryos of a single pod of *P. pictus* wriggled out one after another within several minutes. After shedding the membrane the young grasshoppers stood upright and were able to jump away. *P. pictus* was reared in captivity from 1st instar to adult; the moulting time was noted. During each moult it held firmly to the wooden stick placed in the chamber and then wriggled out of the skin. The process lasted for 4-7 hours. The nymphs were more susceptible to infestation during moulting a variety of flies and ants. Red mites were often seen as external parasites on *P. pictus*.

Wing pads of first to third instar hoppers were borne saddle-like over the thorax. Wing pads of fourth and fifth instar hoppers were pointed backward over the abdomen and differed only in size. In the fourth instar, wing pads were relatively small and extended only to the first abdominal segment, while in the fifth instar they were large and extended past the second abdominal segment. During the final moult, when nymphs moult to an adult, the freshly formed wings looked pinkish red, delicate, and shorter than the actual wings of the adult. Within 2-2½ hours they appeared as long as in complete adult stage, showing the blue, green, yellow mosaic pattern with a brown end, and stronger (strong enough to fly) than the imago.

The new adult had fully functional wings but was not immediately ready to reproduce. The female had a pre-oviposition period of 15-30 days during which she increased in weight till the first batch of eggs matured.

Individual variation in the duration of instars within Group II was not statistically significant (P=0.05). The variation in period of each instar, total nymphal periods and number of instars between males and females of Group II was statistically significant (P=0.05).

The entire nymphal period averaged 25 days for males and 34 days for females. Each instar took four to five days to complete development except for the last instar, which took seven to ten days. Adult longevity of males averaged 266 days, and that of females 273 days (Table 2).

4. Mating and Oviposition

Caged females of *P. pictus* usually became receptive to courting males 2-5 days after their final moult, or even sooner when crowded with 6 males in a growth chamber. The males can copulate 5-10 days after the final ecdysis. Males attracted females both visually and acoustically, by short flights, flashing their brightly coloured wings, snapping them together, or both, producing a distinct sound (crepitation). Males also attracted females by stridulation (scraping the hind femur against the forewing). Female body coloration faded after copulation. Table 3 includes number of clasping males (1-5 individuals), number of copulations of females before oviposition (2-17), and average mating time (3-14 hrs).

Abdominal ends of gravid females bend in an angle and at that stage they were more lethargic. Oviposition started 15-30 days after the final moult and 13-25 days post-copulation for all 15 females, and was stimulated by wetting the sand.

Female had two pairs of valves (triangle shapes) at end of abdomen to dig in sand during egg laying. Each female laid one or, rarely, two egg pods, with an average of 126 eggs per egg pod. The egg pods were laid 2-3 inches deep in the soil bed that the female deposited from her abdomen. The egg-pod of *P. pictus* was elongated, soft, fragile and bent near the base. A stout pod forms from frothy glue and soil surrounding the eggs; froth was lacking between the eggs. The frothy material probably protected the eggs from parasites, desiccation and mechanical hazards.

Eggs (Fig. 3) varied in size, colour, and shell sculpturing. Eggs were cylindrical, elongated and some were

Table 2: Moulting periods of *P. pictus* in Group II, reared at a temperature of 78.8 -89.6 °F (26-32 °C) and 30-40% relative humidity, and fed on diet of fresh green leaves of *C. gigantea*

Stage (n=42)	Male (in days)	Female (in days)
Instar 1	4.0	4.0
Instar 2	4.3	3.8
Instar 3	4.1	3.9
Instar 4	5.0	4.5
Instar 5	7.7	7.3
Instar 6	-	10.1
Total nymphal period	25.1	33.6
Average adult longevity	266	273

Table 3: Copulation behaviour in *P. pictus* of Group II (n=15), which includes number of clasping males/mating, number of times female copulates before oviposition and average copulation/mating in hours

Sr. No.	No. of clasping males/mating	No. of times female copulates before oviposition	Mean±SD copulation/ mating time (in hrs)
1	3	11	6±1.37
2	4	12	9±1.41
3	2	5	12±0.74
4	1	7	10±0.39
5	5	9	8±1.26
6	4	2	3±1.98
7	3	4	10±0.45
8	4	9	9±1.47
9	2	10	12±0.10
10	2	15	11±0.50
11	1	17	14±0.80
12	2	6	9±1.56
13	3	8	7±0.91
14	3	7	6±2.08
15	4	13	12±0.34
Range	1-5 individuals	2-17 times	3-14 hrs
Mean± SD	2.87±1.187	9±4.123	9.2±2.883

Fig. 3: *P. pictus* eggs separated from egg pod

slightly bent. They were yellow to dark brown in colour. The egg-wall showed a mosaic hexagonal pattern.

Maximum egg pod length was 7.89 cm; mean egg breadth and length was 7.59 mm and 0.9 mm respectively. After oviposition, the blue-green coloration of the body stripes of the female changed to light green.

B. Growth parameters

Lengths and weights

Using linear measurements of the body, linear relationships have been demonstrated graphically in *P. pictus* between the body weight and length (Figs 4-7). Male and female grew to a maximum adult size of 7.55 ±0.83 cm and 11.23 ±1.41 cm in length, and 3.19 ±0.41 gm and 6.73 ±0.51 gm of wet weight, respectively, under laboratory conditions. Whereas, males and females collected from natural habitat (Group I) had a maximum size of 6.17 ±0.76 cm, 8.32 ±0.96 cm in length, and 2.23 ±0.24 gm, 4.73 ±0.47gm of wet weight, respectively.

The variation in weight as well as length of female grasshoppers in Groups I and II was statistically significant (Table 4, $P \leq 0.05$), whereas the variation in mean length of the male grasshoppers between both the groups was not significant. The variation in the mean weight of males in both the habitats is statistically significant (Table 4, $P \leq 0.05$). Females in both the groups were larger than males. Lengths as well as weights of adult females were greater, statistically, than those of the adult males (Table 4) at $P \leq 0.05$. Fig. 8 shows

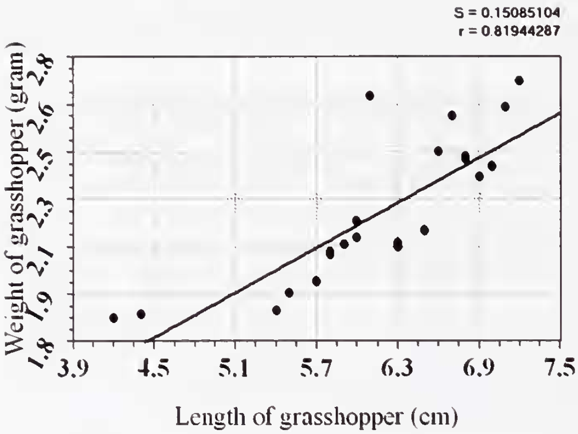

Fig. 4: Regression graph for Group I 'Male'

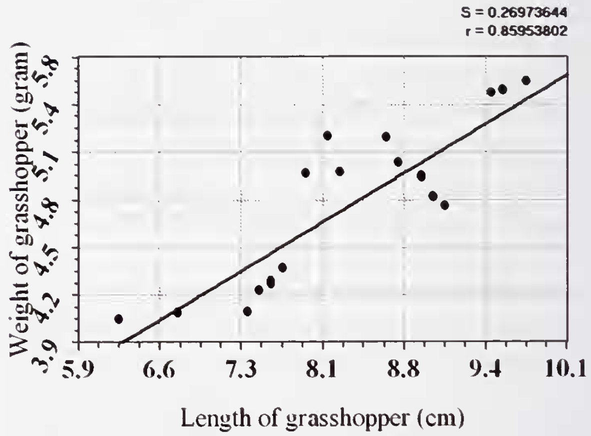

Fig. 6: Regression graph for Group I 'Female'

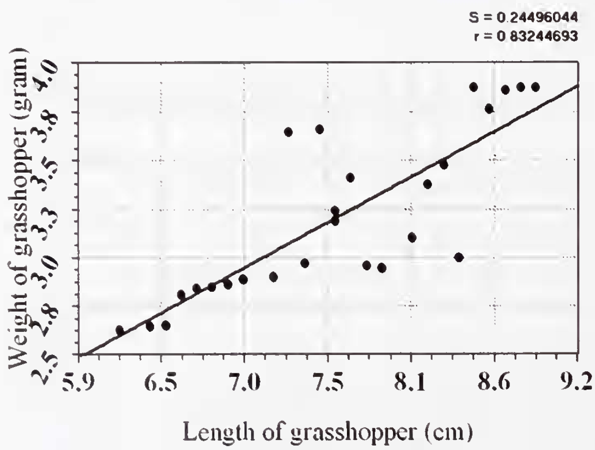

Fig. 5: Regression graph for Group II 'Male'

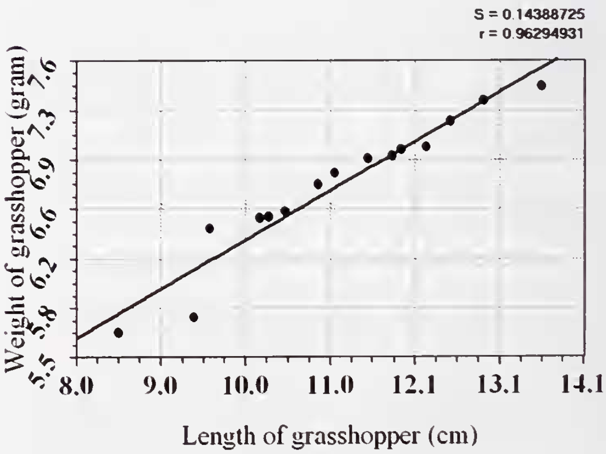

Fig. 7: Regression graph for Group II 'Female'

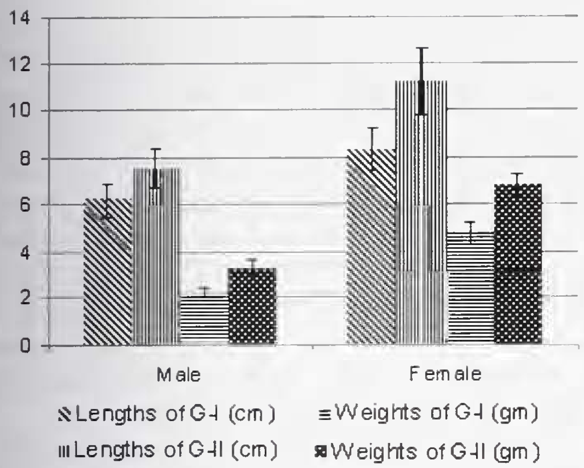

Fig. 8: Variation in mean length (cm) and weight (gm) with SD for Groups I and II

variations in mean length and weight with standard deviation of all groups (Group I and II).

In any organism body length and weight are partially correlated with each other. In the present study, there was perfect positive correlation in Group II 'females' (r= 0.962949), and partial positive correlation (r= 0.859538, 0.832446, 0.81944 respectively) in Group II 'males', and Group I 'males and females' (Table 5).

DISCUSSION

In the present investigation, behavioural study, growth pattern with the length and weight correlations are studied together. The biology and behaviour of *P. pictus* were described by some entomologists and zoologists in various parts of India and Pakistan (Delvi and Pandian 1972a; Sayed *et al.* 1994; Singhal 1976; Parihar 1971; Butani 1975). However, many of these studies are limited either to reproduction or food consumption and assimilation rates.

We observed that laboratory fed adult males *P. pictus* were more active than their counterparts in natural conditions. This is probably because adults in natural habitat stick to the stems of host plant sucking the latex (as nymphs have voraciously defoliated the leaves), and the laboratory reared *P. pictus* were fed only on fresh leaves and the stems were not available for them to hold onto. Further observation was that nymphs eject the secretion forcibly several times in the direction of a disturbance, whereas in adults the secretion flows down the sides of the body along lateral grooves into the spiracles of the second abdominal segment where it mixes with air to form a repellent froth which was in accordance with the observations reported by Qureshi and Ahmad (1970). Perception of the odour of *P. pictus* by human volunteers was studied for the first time. The study showed that the odour of *P. pictus* was offensive and unpleasant. A similar description was used for the odour of related grasshoppers by Whitman (1990). The study has also shown that the production of odour was maximum in adults. Gupta (1978) has reported that sex pheromones are secreted in metathoracic and first 2 segments of the abdomen by female *P. pictus*. Gillott (2003) reported that secretions of accessory glands in male grasshopper include noxious chemicals and various bio-molecules. Adult *P. pictus* also produces appreciable volume of defensive secretion (Qureshi and Wahid 1969). Production of pheromones and defensive secretion might have contributed to the volume of odour. We have noted repellent and irritant responses of the defensive secretion of *P. pictus* on human beings with rashes and allergic reaction on skin. Qureshi and Wahid (1969) have described repellent, irritant and lethal effects of the defensive secretion of *Poecilocerus pictus* in laboratory experiments on fish, reptiles, birds and mammals, but not on human beings.

It was clearly evident from our results that feeding rate of the second instar nymphs in both the sexes was highest (2.88 gm/day in males and 5.15 gm/day in female); female nymphs were observed to consume twice the amount of food than male nymphs. During the period of investigation average food consumption of adult male and female was 2 gm and 4.45 gm/day/individual respectively. Contradicting our results, Delvi and Pandian (1972b) reported that adult males consume more food than adult females, i.e., 904 mg/gm body weight per day in males and, 662 mg/gm body weight per day in females. Sayed *et al.* (1994) reported that *P. pictus* (feeding on *Calotropis* sp.) adult female consumes 9.37 gm

Table 4: Lengths and weights of *P. pictus* in Groups I and II

Group	Mean length	Mean weight	No. of observations
Group I Male	6.17±0.76 (NS)	2.23±0.24*	n=25
Group II Male	7.55±0.83 (NS)	3.19±0.41*	n=27
Group I Female	8.32±0.96**	4.73±0.47**	n=20
Group II Female	11.23±1.41**	6.73±0.51**	n=15

*: Statistically Significant, **: Highly Significant, NS: Statistically not significant

Table 5: Correlation Coefficient between mean lengths and weights in Groups I and II

Group	Correlation Coefficient
Group I Male	0.81944
Group II Male	0.832446
Group I Female	0.859538
Group II Female	0.962949

food plant per day. Singhal (1976) studied consumption and assimilation rates and reported higher consumption and assimilation rates in males than females. However, consumption rates are higher in females than in males in our observation, probably because they have to prepare themselves for oviposition. Photopositive responses and positive reaction towards the odour of food is evident in our results and is in agreement with that reported by Abdullah and Siddiqui (1971). We observed no cannibalism in laboratory reared *P. pictus* which was reported by Parihar (1974).

According to Delvi and Pandian (1972a) and Butani (1975) hatching occurs during March-April, by August the insects undergo six moults to become adult; oviposition occurs during September-October, and death by early December. In our study, hatching extended till August, there were six nymphal stages for females and only five were noted in males at a temperature of 29 ±3°C. The adults survived in healthy conditions till March. Parihar (1971) mentioned six nymphal stages at 30-35 °C, and six or seven stages at 25°C.

The nymphal period in laboratory condition was 25.1 days for males and 33.6 days for females, whereas as mentioned by Butani (1975) the adults appear 4-6 weeks later, i.e., within 28-42 days. The decrease in nymphal periods under laboratory conditions may be probably due to adequate food, temperature and humidity. Muthukrishnan and Delvi (1974) had reported that reduced supplies of *Calotropis gigantea* produce a number of negative effects on *Poecilocerus pictus*, such as heavy mortality (42% at 25% ration of *Calotropis gigantea* against 11% at 100% ration of *Calotropis gigantea*), extension of larval period (from 75 days to 113 days), and an increase in the number of instars (from 6 to 7).

Copulation and oviposition in *Poecilocerus pictus* took place more or less in similar pattern with very few variations as reported by Sheri (1976), Raziuddin *et al.* (1977) and Parihar (1974, 1984). There was a slight degree of variation in number of days in which the males and females become ready for copulation after their emergence as adults, number of clasping males, number of copulations of females before oviposition, average mating time and number as well as structure of egg-pods and egg. In addition, the phenomenon of males attracting females in their reproductive stages by visual and acoustic stimuli was also observed.

The growth efficiency, in our experiments was higher in females as their weights are higher than males in both laboratory conditions and natural habitat. Singhal (1976) worked on growth efficiency ratios, which were higher in females than in males. A female and male grew to a maximum size of 5.1 ±2.3 and 2.7 ±1.8 gm wet weight on the 236th and 218th day of life respectively. Males are correlated with the maximum weight attained (2.6 gm); females attain 5.0 gm in a similar life span (about 265 days at 26 °C) Delvi and Pandian (1972b). Weight gain was higher in laboratory conditions (average 3.19 gm for males and 6.73 gm for females).

The behaviour pattern and life cycle of laboratory reared *P. pictus* was found to be more or less similar to already cited reports. The noteworthy observations in the present study were of longevity of adults and shortening of nymphal periods to 25.1-33.6 days, with the body achieving maximum length and weight resulting in a perfect positive correlation of these parameters.

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